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**Exploration: Allele Frequencies and Sickle Cell Anemia**

**Objective**: To observe how selective forces can change allele frequencies in a population and cause evolution to occur.

**Background**:

Sickle cell anemia was the first genetic disease to be characterized at the molecular level. The mutation responsible for sickle cell anemia is small—just ONE nucleotide of DNA out of the three billion in each human cell. Yet it is enough to change the chemical properties of hemoglobin, the iron and protein complex that carries oxygen within red blood cells.

There are approximately 280 million hemoglobin molecules in each red blood cell (RBC). The protein portion of hemoglobin consists of four globin subunits: two alpha (α) and two beta (β).

These two types of subunits are encoded by the α and β globin genes, respectively. While the binding of oxygen actually occurs at the iron sites, all four globin chains must work together in order for the process to function well.

Sickle cell anemia, also known as sickle cell disease, is caused by a point mutation in the β globin gene. As a result of this mutation, valine (a non-polar amino acid) is inserted into the β globin chain instead of glutamic acid (an electrically charged amino acid). The mutation causes the RBCs to become stiff and sometimes sickle-shaped when they release their load of oxygen.

The sickle cell mutation produces a “sticky” patch on the surface of the β chains when they are not complexed with oxygen. Because other molecules of sickle cell hemoglobin also develop the sticky patch, they adhere to each other and polymerize into long fibers that distort the RBC into a sickle shape.

The sickled cells tend to get stuck in narrow blood vessels, blocking the flow of blood. As a result, those with the disease suffer painful “crises” in their joints and bones. They may also suffer strokes, blindness, or damage to the lungs, kidneys, or heart. They must often be hospitalized for blood transfusions and are at risk for a life-threatening complication called acute chest syndrome. Although many sufferers of sickle cell disease die before the age of 20, modern medical treatments can sometimes prolong these individuals’ lives into their 40s and 50s.

There are two β globin alleles important for the inheritance of sickle cell anemia: A and S.

Individuals with two normal A alleles (AA) have normal hemoglobin, and therefore normal RBCs. Those with two mutant S alleles (SS) develop sickle cell anemia. Those who are heterozygous for the sickle cell allele (AS) produce both normal and abnormal hemoglobin.

Heterozygous individuals are usually healthy, but they may suffer some symptoms of sickle cell anemia under conditions of low blood oxygen, such as high elevation. Heterozygous (AS) individuals are said to be “carriers” of the sickle cell trait. Because both forms of hemoglobin are made in heterozygotes, the A and S alleles are co-dominant.

About 2.5 million African-Americans (1 in 12) are carriers (AS) of the sickle cell trait. People who are carriers may not even be aware that they are carrying the S allele!

In the United States, about 1 in 500 African-Americans develops sickle cell anemia. In Africa, about 1 in 100 individuals develops the disease. Why is the frequency of a potentially fatal disease so much higher in Africa?

The answer is related to another potentially fatal disease, malaria. Malaria is characterized by chills and fever, vomiting, and severe headaches. Anemia and death may result. Malaria is caused by a protozoan parasite (Plasmodium) that is transmitted to humans by the Anopheles mosquito.

When malarial parasites invade the bloodstream, the red cells that contain defective hemoglobin become sickled and die, trapping the parasites inside them and reducing infection.

Compared to AS heterozygotes, people with the AA genotype (normal hemoglobin) have a greater risk of dying from malaria. Death of AA homozygotes results in removal of A alleles from the gene pool. Individuals with the AS genotype do not develop sickle cell anemia and have less chance of contracting malaria. They are able to survive and reproduce in malaria-infected regions. Therefore, BOTH the A and S alleles of these people remain in the population. SS homozygotes have sickle cell anemia, which usually results in early death. In this way, S alleles are removed from the gene pool.

In a region where malaria is prevalent, the S allele confers a survival advantage on people who have one copy of the allele, and the otherwise harmful S allele is therefore maintained in the population at a relatively high frequency. This phenomenon will be examined in the Allele Frequencies and Sickle Cell Anemia Exploration, which relates the change in allele frequency in a population to evolution.

The frequency of the S allele in malaria-infected regions of Africa is 16%. The sickle cell allele is also widespread in the Mediterranean and other areas where malaria is or used to be a major threat to life. In contrast, the S allele frequency is only 4% in the United States, where malaria has been virtually eliminated. Malaria was once common in the United States, but effective mosquito control caused the number of cases to drop. Recently, however, there has been an increase in the number of malarial cases because of increased travel, immigration, and resistance to medication. In Southern California there was a 1986 outbreak of nearly 30 cases of malaria transmitted by local mosquitos!

**Introduction**:

Allele frequency refers to how often an allele occurs in a population. Allele frequencies can change in a population over time, depending on the ‘selective forces’ shaping that population. Predation, food availability, and disease are all examples of selective forces.

Evolution occurs when allele frequencies change in a population!

In this activity, black and white beans are used to represent two alleles of β globin. The BLACK beans represent gametes carrying the β globin A allele, and the WHITE beans represent gametes carrying the β globin S allele. The Gene Pool exists in a region of Africa that is infested with malaria. You are simulating the effects of a high frequency of malaria on the allele frequencies of a population.

**Hypothesis**:

What do you think will happen to the frequencies of the A and S alleles as a result of the presence of malaria? (Will the frequency of A increase or decrease? What about S?) Formulate a hypothesis. Be sure to explain your reasoning.

If\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_then\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_because\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Materials:** 75 black beans, 25 white beans, 5 containers (e.g. paper cups)

**Procedure**:

1. Together with your lab partner, obtain five containers and label them as follows:

1) AA 2) AS 3) SS 4) Non-surviving alleles 5) Gene Pool

2. Place the 75 black and 25 white beans in the Gene Pool container and mix the beans up.

3. Simulate fertilization by PICKING OUT two ‘alleles’ (beans) WITHOUT LOOKING.

4. For every two beans that are chosen from the gene pool, another person will FLIP A COIN to determine whether that individual is infected with malaria.

5. Using the table below, the coin flipper tells the bean picker in which containers to put the beans.

|  |  |  |  |
| --- | --- | --- | --- |
| Genotype | Phenotype | If have malaria (heads)… | If not infected (tails)… |
| AA (Black-Black) | No SCA; susceptible to malaria | Die: place in Non-surviving cup | Live: place in AA cup |
| AS (Black-White) | No SCA; malaria resistance | Live: place in AS cup | Live: place in AS cup |
| SS (White-White) | SCA | Die: place in Non-surviving cup | Live (but for shorter time): place in SS cup |

6. Repeat steps 3–5 until all the beans in the Gene Pool are used up.

7. We are going to pretend SS individuals do not survive to reproduce because treatment is not readily available, move all beans from the SS alleles container into the Non-surviving alleles container.

8. COUNT the number of individual black beans (A alleles) and white beans (S alleles) in the containers labeled AA and AS. These individuals survive to reproduce. RECORD those numbers in the F1 TOTAL SURVIVING ALLELES table.

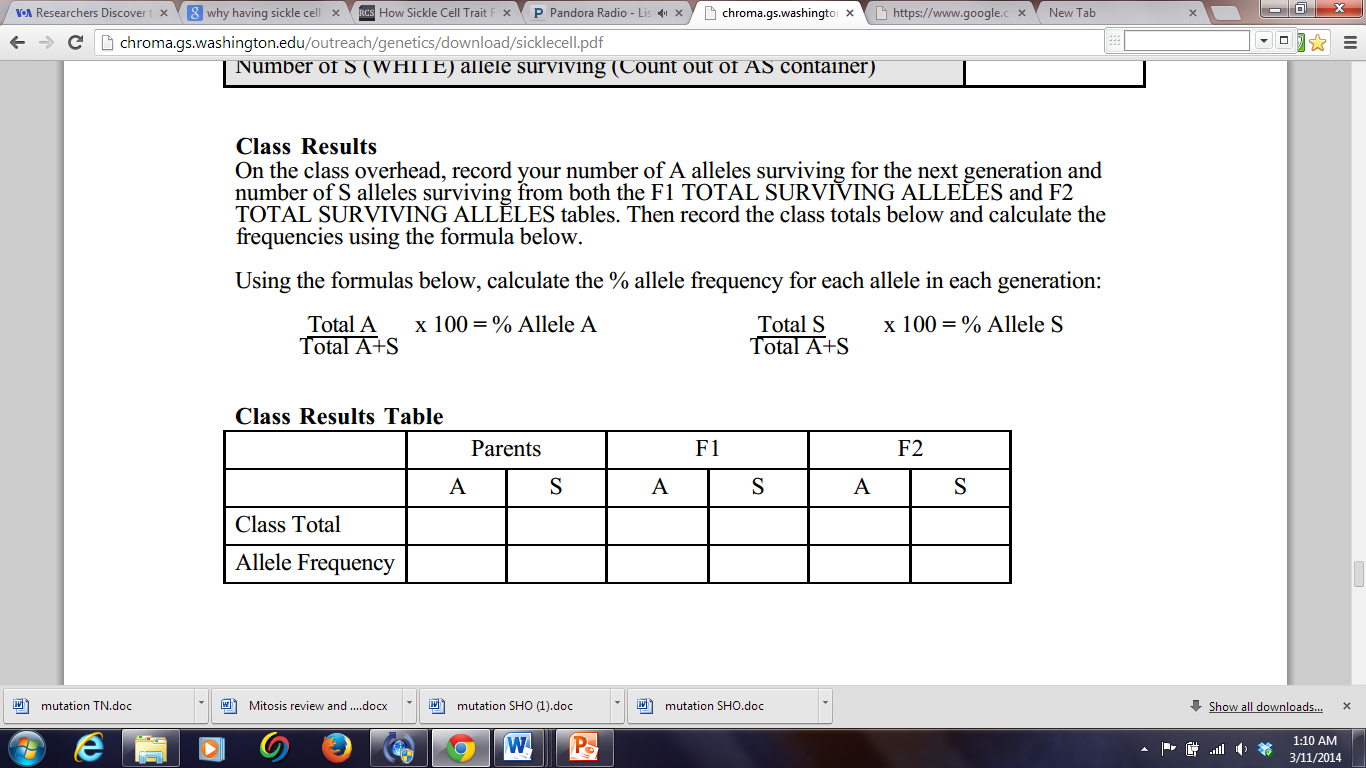
9. Put the surviving alleles back in the gene pool.

10. Repeat steps 3-8 for the F2 generation. Record your results in the F2 TOTAL SURVIVING ALLELES table.

Data Tables:

|  |  |
| --- | --- |
| F1 TOTAL SURVIVING ALLELES | |
| Number of A (BLACK) alleles surviving (count of black beans in AA and AS containers): |  |
| Number of S (WHITE) alleles surviving (count of white beans in AS containers): |  |

|  |  |
| --- | --- |
| F2 TOTAL SURVIVING ALLELES | |
| Number of A (BLACK) alleles surviving (count of black beans in AA and AS containers): |  |
| Number of S (WHITE) alleles surviving (count of white beans in AS containers): |  |

Using the formulas below, calculate the % allele frequency for each allele in each generation: 

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Parents | | F1 | | F2 | |
|  | A | S | A | S | A | S |
| Number of Alleles | 75 | 25 |  |  |  |  |
| Allele Frequency | 75% | 25% |  |  |  |  |

**Analysis and Conclusion**:

1. What do the black and white beans represent in this simulation? What does the coin represent?

2. What do you think “allele frequency” means? How are allele frequencies related to evolution?

3. What are the “selective forces” in this simulation (the forces changing the allele frequencies)?

4. What was the general trend you observed for Allele A over the three generations (did it increase or decrease)? What was the general trend for Allele S over time? Was your hypothesis supported?

5. Do you anticipate that the trends in question 4 will continue for many generations? Why or why not?

6. Since few people with sickle cell anemia (SS) are likely to survive to have children of their own, why hasn’t the mutant allele (S) been eliminated? (Hint: what is the benefit of keeping it in the population?)

7. Why is the frequency of the sickle cell allele so much lower in the United States than in Africa?

8. Scientists are working on a vaccine against malaria. What impact might the vaccine have in the long run on the frequency of the sickle cell allele in Africa? (Would it increase or decrease? Why?)